

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
12 September 2003 (12.09.2003)

PCT

(10) International Publication Number  
**WO 03/073981 A2**

- (51) International Patent Classification<sup>7</sup>: **A61K**
- (21) International Application Number: PCT/SE03/00347
- (22) International Filing Date: 4 March 2003 (04.03.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
0200667-4 5 March 2002 (05.03.2002) SE  
10/092,919 8 March 2002 (08.03.2002) US
- (71) Applicant (*for all designated States except US*): **PHARMASURGICS AB** [SE/SE]; c/o A+Science Invest AB, Gardatorget 1, SE-412 50 Göteborg (SE).

- (72) Inventor; and
- (75) Inventor/Applicant (*for US only*): **OLMARKER, Kjell** [SE/SE]; Gustavsgatan 35, S-431 66 Mölndal (SE).
- (74) Agent: **AWAPATENT AB**; Box 5117, S-200 71 Malmö (SE).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: NOVEL OF CYTOKINE INHIBITORS

(57) Abstract: The use of substance that inhibits a pro-inflammatory cytokine, such as TNF or IL-1, for the production of a pharmaceutical composition for improving wound healing is disclosed. Also a method for improving wound healing wherein a therapeutically effective amount of a substance that inhibits a pro-inflammatory cytokine is administered to a patient in need of said treatment is disclosed.

WO 03/073981 A2

NOVEL USE OF CYTOKINE INHIBITORSField of the invention

The present invention relates to pharmaceutical compositions and methods for improving wound healing.

Background of the invention

5 Wound healing is a positive physiological reaction that may restore anatomy and function of various tissues after trauma. The trauma may be accidental, the result of surgical intervention or the effect of a disease or genetic condition. The ideal end result of wound healing should be to restore the tissues to the situation before the trauma. Wound healing may be delayed or incomplete due to various independent factors. This may lead to chronic conditions with impaired restoration and function of the injured tissue. Many factors delaying the wound healing may be inhibited by specific treatment. For instance, infections may be treated by antibiotics, reduced blood flow may be treated by compression bandages and oxygen therapy and seromas may be evacuated by drainage. One additional way to facilitate wound healing would be to reduce the scar formation. In all cases of wound healing, however, it would be desirable to enhance the inborn properties of tissues to heal by interfering with the wound healing process per se. A method for controlling and improving wound healing would be of a great value in most cases of posttraumatic or post surgical wound healing.

Summary of the invention

25 Based on the knowledge derived from the literature the inventor assessed the efficacy of improving wound healing by administration of TNF in a laminectomy model on the rat (see the Comparative Example below). To his surprise, he found, contrary to what could be expected, that the wound healing was significantly impaired in the rats exposed to TNF.

30 Since administration of TNF increased scar formation and also negatively influenced the wound healing per se, the inventor realized that the *in vitro* data acquired in experimental settings regarding fibroblast regulation are

not applicable *in vivo*, and that these findings had to be re-evaluated in light of the *in vivo* situation.

The cytokine network is complex and what may seem to be evident from an *in vitro* setting may often prove not to be applicable in the *in vivo* setting. The *in vivo* situation at the area of wound healing comprises a vast number of known and unknown substances that may interact in ways not present *in vitro*. Administration of a cytokine in one concentration may have an effect that is counteracted by administration of the same cytokine in a higher dose due to synergistic inhibition and stimulation between the various cytokines as well as physiological inhibition of its release from adjacent cells.

It is generally assumed that the inflammatory reaction seen in tissues undergoing wound healing is a positive event that is necessary for normal wound healing to occur. However, the inventor assumed that this might be a misleading conclusion based on the findings of the comparative example (see below). Various biological substances regulate the activity of specific cells during physiological processes. This may be assumed to be true also regarding wound healing. One component of normal wound healing is the regeneration of injured cells at the site of wound healing. Regeneration is usually promoted by various growth factors. Under certain circumstances, pro-inflammatory cytokines may counteract the effects elicited by the growth factor in a way that there is a balance between stimulation (growth factors) and inhibition (pro-inflammatory cytokines). The inventor assumed that this might be the case also at wound healing. A feasible way to shift the balance of stimulation and inhibition by pharmacological means would be to either enhance the level of stimulation or to reduce the inhibition. Since the comparative example (see below) clearly indicated that administration of TNF reduced the wound healing the author assumed that inhibition of the same or other substances with similar action instead might improve wound healing.

In the literature it has been recognized that fetal tissues heal with emphasis of regeneration of the injured tissue with no or little scar formation. In contrast, adult tissues instead may result in scar formation that may dominate over tissue regeneration. Since the inflammatory reaction in fetal tissues during wound healing is less pronounced than during adult wound healing one might assume that reduction of the inflammation *per se* might be beneficial for the wound healing.

The inflammation at the site of wound healing induces an increased blood flow in the wound healing area. The inflammation also induces an activation of adhesion molecules that, together with a simultaneous increase in vascular permeability, may facilitate the migration of inflammatory cells to the wound healing site. Inflammatory mediators also have leucotactic or chemotactic properties, i.e. attract white blood cells to the area of wound healing. Two important inflammatory mediators responsible for this leucotaxis are TNF and IL-1. TNF and IL-1 synergistically with other chemokines stimulate this local inflammatory response. Since the main contribution of inflammatory cytokines to the site of wound healing is delivered by invading leucocytes it would be useful to limit the number of leucocytes by inhibiting the migration of these cells. Since pro-inflammatory substances are mainly responsible for the migration of the leucocytes to the wound healing site this should be achieved by inhibiting the activity of such substances.

The inventor therefore assumed that a more feasible way to improve wound healing than previously suggested was to reduce the activity of pro-inflammatory substances, which he also later found to be true. This both prevents cell migration to the site of wound healing and may shift the balance of stimulation and inhibition of tissue regeneration in favor of stimulation. Since TNF and IL-1 are responsible for both these mechanisms, the most efficient way to improve wound healing is to inhibit the action of these two pro-inflammatory cytokines or other pro-inflammatory cytokines. This was also confirmed in the example displayed below.

The characterizing features of the invention will be evident from the following description and the appended claims.

#### Detailed description of the invention

As discussed above, and further demonstrated in the Example below wherein administration of infliximab to pigs with a standardized laminectomy is discussed, the inventor found, contrary to what could be expected from existing literature, that inhibition of pro-inflammatory cytokines is an efficient way to control wound healing. Such pro-inflammatory cytokines are tumor necrosis factor (TNF), interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 12 (IL-12), interleukin 15 (IL-15), interleukin 17 (IL-17), interleukin 18 (IL-18), granulocytes-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), monocyte chemotactic

protein-1 (MCP-1), macrophage inflammatory protein 1 (MIP-1), RANTES (regulated upon activation, normal T-cell expressed, and presumably secreted), epithelial cell-derived neutrophil attractant-78 (ENA-78), oncostatin-M (OSM), fibroblast growth factor (FGF), platelet derived growth factor (PDGF),  
5 and vascular endothelial growth factor (VEGF); and in particular TNF (also called TNF- $\alpha$ ) and IL-1 (including both IL-1  $\alpha$  and IL-1  $\beta$ ).

The suggested treatment is applicable at all kinds of surgery. It may also be used after traumatic tissue injury. Tissue injury may also be the result of toxic influence, as the result of reduced blood flow due to vascular disease, or  
10 as the result of a thermic injury, and the treatment according to the invention is applicable also for these three latter conditions.

For the purpose of this disclosure, the terms "blocking agent", "blocking substance", "inhibitor" and "antagonist" may be used interchangeably.

As stated above, inhibition of a pro-inflammatory cytokine is useful for  
15 improving wound healing. This inhibition is possible to achieve by any suitable cytokine inhibitor, such as available pharmacological compositions.

Persons skilled in the art are well aware of what is intended by a pro-inflammatory cytokine. For the purpose of this disclosure, it may, however, be further clarified that the expression "a pro-inflammatory cytokine" relates to  
20 any substance from the cytokine family that posses one or more of the following specific mechanisms of action: <sup>1)</sup> increasing vascular permeability, <sup>2)</sup> attracting white blood cells (leucotaxia or chemotaxia), <sup>3)</sup> activating macrophages, and <sup>4)</sup> recruiting macrophages to the site of wound healing. These effects may be assessed for each individual substance by use of the assays disclosed below. "A substance that inhibits a pro-inflammatory cytokine" as it is  
25 used herein thus relates to a substance that may block one or more of the four listed effects in the assays disclosed below. However, due to differences between species, one may also translate findings from the experimental setting to the human situation. For instance, if a monoclonal antibody with specificity  
30 towards a specific cytokine of a certain species inhibits the action of the cytokine in one of the three ways disclosed below in that specific species, one may assume that a monoclonal antibody, with specificity towards the human version of the cytokine, may inhibit this cytokine in the human situation.

<sup>1)</sup> Assay for increase of vascular permeability: A golden hamster, weighing  
35 65-100 g, is anaesthetized with a mixture of Apozepam® (Diazepam 5 mg/ml Apothekarnes Laboratorium, Oslo, Norway) and Mebumal Vet®

(Pentobarbital 60 mg/ml, NordVacc Vaccin AB, Malmö, Sweden) volume ratio 10:1. An initial dose of 0.3 ml is given intraperitoneally. Additional injections of 0.1-0.4 ml are administered each 30 minutes. The hamster is placed on a heated (37° C) perspex plate, and the right cheek-pouch is everted over a translucent rubber plate and covered with plastic film in order to prevent reduction in blood flow rate due to direct exchange of oxygen. An injection of 0.3 ml of FITC-Dextran (mw 150.000, 25 mg/ml, Sigma, St Louis, USA) is made in the femoral vein for fluorescence vital microscopic observations of macromolecular extravascular leakage. Temperature and humidity is controlled by irrigation of saline at 37°C. An injection of approximately 0.02 ml of a suitable concentration of the substance to be tested is made between the two layers of the cheek-pouch using a thin injection needle (diameter 0.4 mm). The same volume of saline is performed in an adjacent part of the cheek-pouch at a distance from the other injection site sufficient to eliminate the risk of communication between the saline and the tested substance within the cheek-pouch. The injection procedures are carried out under a stereomicroscope to minimize mechanical damage to the microvessels. Microvascular reactions are studied for 60 minutes at various magnifications, using fluorescence microscopic techniques (Leitz, Wetzlar, Germany). A pro-inflammatory cytokine as defined according to the present invention induces a leakage of the fluorescent macromolecule FITC-dextran. A similar leakage should not be observed at the site injected by saline.

<sup>2)</sup> Assay for leucotaxia or chemotaxia: A pig, bodyweight 25-30 kg, is anaesthetized with an intramuscular injection of 20 mg/kg bodyweight of Ketalar® (ketamine 50 mg/ml, Parke-Davis, Morris Plains, New Jersey) and an intravenous injection of 4 mg/kg bodyweight of Hypnodil® (methomidate chloride 50 mg/ml, AB Leo, Helsingborg, Sweden) and 0.1 mg/kg bodyweight of Stresnil® (azaperon 2 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). Anesthesia is maintained by additional intravenous injections of 2 mg/kg bodyweight of Hypnodil® and 0.05 mg/kg bodyweight of Stresnil®. One ml of a fluid containing a sufficient concentration of the substance to be tested is placed, in a suitable concentration locally in its natural form, in slow-release preparations or by continuous administration by osmotic mini-pumps, in a specially designed titanium-chamber. The chamber is 5 mm high and has a diameter of 15 mm. The top could be dismantled and is perforated with 18 holes, each with a diameter of 1.4 mm. The chamber, together with one chamber with

the same volume of saline, is placed subcutaneously in the lumbar region through separate incisions, with no communication between the chambers. After 7 days the pig is reanaesthetized similar to the first procedure. The chambers are harvested and the content of the chamber is placed in a test-tube together with 1 ml of Hanks' Balanced Salt Solution (Life Technologies, Paisley, Scotland). From this suspension, 100 µl is used to wash out the chamber for remaining cells. This procedure is repeated 5 times. The test-tube is then shaken for 15 seconds. A total of 25 µl of the suspension and 25 µl of Türk's staining medium (Sigma, St Louis, USA) are mixed and placed in a chamber of Bürker. The total number of leukocytes in each chamber is determined using light microscopy. The chamber with a pro-inflammatory cytokine as defined according to the present invention then contains significantly more white blood cells than the chamber with only saline.

<sup>3)</sup> Assay for activation of macrophages: A macrophage cell line is bought and cultured according to the description of the manufacturer. Examples of useful cell lines are; DH-82 from ECACC, Salisbury, Wiltshire, Great Britain; ACC288, ACC269 or ACC416 from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ); or ICLC ATL98011 from Institute of Pharmacological Sciences, Milan Italy. The cells are cultured in multiple-well culture plates. The substance to be tested is applied to the culture-wells in various concentrations. After incubation for 6-72 hours, aliquots of the culture media (25-50 µl) of the culture media are used for assays. Assays of TNF and IL-8 and Nitric oxide (NO) are performed using commercially available assays and the results are compared with assays from culture media without the addition of the substance to be tested. A pro-inflammatory cytokine as defined according to the present invention induces significantly higher levels of one or more of TNF, IL-8 or NO in the culture media compared to culture media without the tested substance.

<sup>4)</sup> Assay for recruitment of macrophages to the site of wound healing:  
Rats are anaesthetized with a standardized combination of pentobarbital and diazepam. The skin on the back is shaved. A 3 cm long midline incision is made in the skin and in the underlying muscle. The substance to be tested is applied in a suitable concentration locally in its natural form, in slow-release preparations or by continuous administration by osmotic mini-pumps. In control experiments, the same amount and administration of saline is executed. The skin is sutured. After 1-4 weeks the rat is re-anaesthetized and the area of

wound healing in the skin and in the muscle is harvested and processed for immunohistochemistry. Commercially available antibodies for macrophage specific CD-molecules (e.g. CDw17, CD23, CD25, CD26, CD64, CD68, CD69, CD71, CD74, CD 80, CD88, CD91 and CD105) are used to visualize the presence of macrophages in the healing tissues. The number of macrophages is then found to be significantly higher in the healing tissue when exposed to the tested substance than in tissues exposed to saline control.

Inhibition of pro-inflammatory cytokines: An inhibitor of a pro-inflammatory cytokine as defined according to the present invention will reduce the effects of the pro-inflammatory cytokine in one or more of the four assays above, i.e. increase of vascular permeability, leucotaxia and activation or recruitment of macrophages, and/or it will have an inhibitory effect on the recruitment of macrophages in the assay for inhibition of recruitment of macrophages disclosed below.

<sup>5)</sup> Assay for inhibition of recruitment of macrophages to site of wound healing: Rats are anaesthetized with a standardized combination of pentobarbital and diazepam. The skin on the back is shaved. A 3cm long midline incision is made in the skin and in the underlying muscle. The skin is sutured. The animal receives treatment by a cytokine inhibitor in a suitable concentration and form of administration. Control animals receive no treatment. After 1-4 weeks the rat is re-anaesthetized and the area of wound healing in the skin and in the muscle is harvested and processed for immunohistochemistry. Commercially available antibodies for macrophage specific CD-molecules (e.g. CDw17, CD23, CD25, CD26, CD64, CD68, CD69, CD71, CD74, CD 80, CD88, CD91 and CD105) are used to visualize the presence of macrophages in the healing tissues. The number of macrophages is then found to be significantly lower in the healing tissue after treatment with the cytokine inhibitor than in control animals.

The term "patient", as it is used herein, relates to any human or non-human mammal in need of treatment according to the invention.

Wound healing that may be improved according to the present invention is healing after any kind of tissue injury such as following surgery, by traumatic tissue injury, tissue injury resulting from toxic influence, or thermic injury, or as the result of reduced blood flow due to vascular disease. The expression "improved wound healing" and similar expressions used herein are intended to relate to both improvement of the time for a wound to heal and to

improvement of the quality of the wound. By improved wound healing is thus intended wound healing that is improved, enhanced and/or facilitated by use of the substances according to the present invention, when compared to a similarly wound not treated with a compound according to the invention. A wound is a state where the normal anatomy of a structure has been destructed. This could encompass a separation of the tissue into two wound surfaces as in a traumatic wound induced by a knife or removal of a tissue as in an abrasion wound. Wounds may also be achieved by metabolic processes due to reduced nutritional supply such as diabetic, leg and decubitus ulcer as well as gastric ulcers, or by exposure to toxic compounds or thermal injury. Wounds may also be induced by surgery. Wound healing is the physiological mechanism to restore the injured tissue to its original condition and anatomy. The success of wound healing can be measured both as the time for a specific wound to heal and the quality of the wound healing. Healing after a traumatic cut wound would encompass the adhesion of the two separated wound surfaces. This is a time dependant process and a substance promoting improved wound healing would typically induce a higher degree of adhesion in a wound at a given time-point following the formation of the wound than a wound not exposed to substance. This is further illustrated in the example below, wherein administration of infliximab provided a higher degree of adhesion than in wounds in pigs without infliximab administration after one week. In this example, infliximab was found to improve wound healing. In cases with abrasion injury, the improved wound healing would be defined as a higher degree of re-epithelialization of the abraded surface in wounds following administration of a substance promoting wound healing than in wounds without administration of such a substance. Improved wound healing is characterized as better data for a wound treated with a substance according to the invention, as compared to a non-treated wound or a wound treated with placebo, regarding clinical appearance, biomechanical testing, histological analyses of biopsy material, high resolution ultra sound scanning and/or other techniques to evaluate degree of wound healing.

The term "treatment" used herein relates to both treatment in order to cure or alleviate a disease or a condition, and to treatment in order to prevent the development of a disease or a condition. The treatment may either be performed in an acute or in a chronic way.

There are several different types of inhibitors of pro-inflammatory cytokines that may be used according to the invention:

- Specific TNF blocking substances, such as
  - Monoclonal antibodies, e.g. infliximab, CDP-571 (Humicade<sup>TM</sup>), D2E7, and CDP-870;
  - Soluble cytokine receptors, e.g. etanercept, lenercept, pegylated TNF-receptor type I, TBP-1
  - TNF-receptor antagonists
  - Antisense oligonucleotides; e.g. ISIS-104838;
- Non-specific TNF blocking substances, such as:
  - MMP inhibitors (i.e. matrix metalloproteinase inhibitors, or TACE-inhibitors, i.e. TNF Alpha Converting Enzyme-inhibitors)
    - Tetracyclines, for example Doxycycline, Lymecycline, Oxitetracycline, Tetracycline, Minocycline and synthetic tetracycline derivatives, such as CMT, i.e. Chemically Modified Tetracyclines;
    - Prinomastat (AG3340)
    - Batimastat
    - Marimastat
    - KB-R7785
  - TIMP-1, TIMP-2, adTIMP-1 (adenoviral delivery of TIMP-1), adTIMP-2 (adenoviral delivery of TIMP-2)
  - Quinolones, for example Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin and Temafloxacin;
  - Thalidomide derivatives, e.g. SelCID, i.e. Selective Cytokine inhibitors, such as thalidomide derivative, for example CC-1088, CDC-501, CDC-801, and Linomide (Roquinex®);
  - Lazaroids; nonglucocorticoid 21-aminosteroids such as U-74389G (16-desmethyl tirilazad) and U-74500
  - Prostaglandins; Iloprost (prostacyclin)
  - Cyclosporin
  - Pentoxifyllin derivatives
  - Hydroxamic acid derivatives

- Phosphodiesterase I, II, III, IV, and V-inhibitors; CC-1088, Ro 20-1724, rolipram, amrinone, pimobendan, vesnarinone, SB 207499 (Ariflo®)
- Melancortin agonists; HP-228
- Other TNF blocking substances, such as:
  - 5 - Lactoferrin, and peptides derived or derivable from lactoferrin such as those disclosed in WO 00/01730
  - CT3
  - ITF-2357
  - PD-168787
  - 10 - CLX-1100
  - M-PGA
  - NCS-700
  - PMS-601
  - RDP-58
  - 15 - TNF-484A
  - PCM-4
  - CBP-1011
  - SR-31747
  - AGT-1
  - 20 - Solimastat
  - CH-3697
  - NR58-3.14.3
  - RIP-3
  - Sch-23863
  - 25 - Yissum project no. 11649
  - Pharma projects no. 6181, 6019 and 4657
  - SH-636
- Specific IL-1 $\alpha$  and IL-1 $\beta$  blocking substances, such as:
  - Monoclonal antibodies;
  - 30 - Soluble cytokine receptors;
  - IL-1 type II receptor (decoy RII)
  - Receptor antagonists; IL-1ra, (Orthogen®, Orthokin®)
  - Antisense oligonucleotides;
- Non-specific IL-1 $\alpha$  and IL-1 $\beta$  blocking substances, such as
  - 35 - MMP inhibitors (i.e. matrix metalloproteinase inhibitors),

- Tetracyclines, for example Doxycycline, Trovafloxacin, Lymecycline, Oxitetracycline, Tetracycline, Minocycline, and synthetic tetracycline derivatives, such as CMT, i.e. Chemically Modified Tetracyclines;
- 5 · Prinomastat (AG3340)
- Batimastat
- Marimastat
- KB-R7785
- TIMP-1, TIMP-2, adTIMP-1, adTIMP-2
- 10 - Quinolones (chinolones), for example Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin, Temafloxacin;
- Prostaglandins; Iloprost (prostacyclin);
- 15 - Phosphodiesterase I, II, III, IV, and V-inhibitors; CC-1088, Ro 20-1724, rolipram, amrinone, pimobendan, vesnarinone, SB 207499.
- Specific IL-6 blocking substances, such as:
  - Monoclonal antibodies;
  - Soluble cytokine receptors;
  - 20 - Receptor antagonists;
  - Antisense oligonucleotides;
- Non-specific IL-6 blocking substances, such as:
  - MMP inhibitors (i.e. matrix metalloproteinase inhibitors)
    - Tetracyclines, for example Doxycycline, Lymecycline, Oxitetracycline, Tetracycline, Minocycline, and synthetic tetracycline derivatives, such as CMT, i.e. Chemically Modified Tetracyclines;
    - 25 · Prinomastat (AG3340)
    - Batimastat
    - Marimastat
    - 30 · KB-R7785
    - TIMP-1, TIMP-2, adTIMP-1, adTIMP-2
  - Quinolones (chinolones), for example Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin, Temafloxacin,
  - 35 - Prostaglandins; Iloprost (prostacyclin)

- Cyclosporin
- Pentoxifyllin derivatives
- Hydroxamic acid derivatives
- Phosphodiesterase I, II, III, IV, and V-inhibitors; CC-1088, Ro 20-1724, rolipram, amrinone, pimobendan, vesnarinone, SB 207499
- Melanin and melancortin agonists; HP-228
- Specific IL-8 blocking substances, such as:
  - Monoclonal antibodies;
  - Soluble cytokine receptors;
  - Receptor antagonists;
  - Antisense oligonucleotides;
- Non-specific IL-8 blocking substances, such as:
  - Quinolones (chinolones), for example Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin, Temafloxacin,
  - Thalidomide derivatives, e.g. SelCID, i.e. Selective Cytokine inhibitors, such as; CC-1088, CDC-501, CDC-801 and Linomide (Roquinex®)
  - Lazaroids
  - Cyclosporin
  - Pentoxifyllin derivatives.

The pharmaceutical composition according to the invention may also comprise other substances, such as an inert vehicle, or pharmaceutical acceptable adjuvants, carriers, preservatives etc., which are well known to persons skilled in the art.

The administration of the TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention should preferably be performed early after injury to limit the inflammatory reaction occurring at the wound healing site. The TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention is administered once or repeatedly until the desired result is obtained. The TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention is administered in a therapeutically effective amount, i.e. an amount that will lead to the desired therapeutical effect and thus lead to an improvement of the patient's condition.

The TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention may be administered in any efficacious way normally used to administer such substances. Thus, the administration may be done both systemically and locally and may be performed before, during  
 5 and/or after all kind of surgical or traumatic tissue injury. The suggested treatment may also be applicable at tissue injury as the result of pathological conditions including vascular disease and toxic influence. The TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention may for example be injected via intra-articular, intravenous (i.v.), intramuscular (i.m.), intraperitoneal (i.p.), intrathecal (i.t.), epidural, intracerebroventricular (i.c.v.) or subcutaneous (s.c.) routes by bolus injections or by continuous  
 10 infusion. They may also be administered orally (per os), e.g. in the form of oral preparations, such as pills, syrups, or lozenges. Furthermore, they may be administered by inhalation or intranasally. Moreover, they may be administered transepidermally, e.g. in the form of topical preparations such as lotions, gels, sprays, ointments or patches. They may also be administered in an irrigation solution or by localized injection. Finally, they may also be administered by  
 15 genetical engineering.

According to one preferred embodiment of the invention, the pharmaceutical composition is formulated as a sustained-release preparation. The substance according to the invention may then, for example, be encapsulated in a slowly dissolving biocompatible polymer.  
 20

Examples of suitable doses for different administration routes are given below.

25	Per os	10-300 mg	
	i.m.	25-100 mg	
	i.v.	2.5-25 mg	
	i.t.	0.1-25 mg	daily - every 3 <sup>rd</sup> month
30	inhalation	0.2-40 mg	
	transepidermally	10-100 mg	
	intranasally	0.1-10 mg	
	s.c.	5-10 mg	
	i.c.v.	0.1-25 mg	daily - every 3 <sup>rd</sup> month
35	epidurally	1-100 mg	

Examples of suitable doses for different TNF inhibitors are given below.

		Preferred dosage	More preferred dosage	Most preferred dosage
5	<u>Lenercept</u>			
	i.v.	5-200	10-100	30-80
		<i>(all doses given in mg for administration once every 4<sup>th</sup> week)</i>		
10	<u>TBP-1</u>			
	i.v.	5-200	10-100	30-80
		<i>(all doses given in mg for administration once every 4<sup>th</sup> week)</i>		
15	<u>CDP-571</u>			
	Humicade®			
	i.v.	1-100	5-10	5-10
		<i>(all doses given in mg/kg body weight for administration as a single dose)</i>		
20	<u>D2E7</u>			
	i.v.	0.1-50	0.5-10	1-10
	s.c.	0.1-50	0.5-10	1-10
		<i>(all doses given in mg/kg body weight for administration as a single dose)</i>		
25	<u>Iloprost</u>			
	i.v.	0.1-2000	1-1500	100-1000
		<i>(all doses given in µg/kg body weight/day)</i>		
	intranasally	50-250	100-150	100-150
		<i>(all doses given in µg/day)</i>		
30	<u>CC-1088</u>			
	Per os	50-1200	200-800	400-600
		<i>(all doses given in mg/day)</i>		

CDP-870

	i.v.	1-50	2-10	3-8
		<i>(all doses given in mg/kg body weight for administration once every 4<sup>th</sup> week)</i>		
5	s.c.	50-600	100-400	100-200
		<i>(all doses given in mg/day)</i>		

Linomide

(Roquinimex®)

	Per os	0.1-25	5-20	10-15
10		<i>(all doses given in mg/kg body weight/day)</i>		

HP-228

	i.v.	5-100	10-50	20-40
		<i>(all doses given in µg/kg body weight)</i>		

ISIS-104838

15	Per os	1-100	10-50	20-50
	S.c.	1-100	10-50	20-50
	i.v.	1-100	10-50	20-50
		<i>(all doses given in mg)</i>		

Ariflo®

20 SB 207499

	Per os	10-100	30-60	30-45
		<i>(all doses given in mg/day)</i>		

KB-R7785

25	S.c.	100-500	100-300	150-250
		<i>(all doses given in mg/kg body weight/day)</i>		

Prinomastat

(AG3340)

	Per os	1-250	5-100	10-50
30		<i>(all doses given in mg for administration twice daily)</i>		

Batimastat

	Per os	1-250	5-100	10-50
		<i>(all doses given in mg for administration twice daily)</i>		

Marimastat

Per os            1-250            5-100            10-50  
*(all doses given in mg for administration  
twice daily)*

5    CDC-501

Per os            50-1200            200-800            400-600  
*(all doses given in mg/day)*

CDC-801

10    Per os            50-1200            200-800            400-600  
*(all doses given in mg/day)*

It is possible to use either one or two or more substances according to the invention in the improvement of wound healing. When two or more substances are used they may be administered either simultaneously or separately.

15    The substances according to the invention may also be administered in combination with other drugs or compounds, provided that these other drugs or compounds do not eliminate the effects desired according to the present invention, i.e. the effect on TNF.

20    It is understood that the response by individual patients to the substances according to the invention or combination therapies, may vary, and the most efficacious combination of drugs for each patient will be determined by the physician in charge.

25    The invention is further illustrated in the Example below, which is only intended to illustrate the invention and should in no way be considered to limit the scope of the invention. The invention is also compared to the stated of the art in the Comparative Example.

Example

30    Six pigs with body weight 25kg underwent a laminectomy of the sacral vertebrae. Three pigs received infliximab (4mg/kg, 100mg in 10 ml sterile water) intravenously and three pigs received an equivalent volume of saline. After one week, wound healing was assessed macroscopically in a blinded fashion using a semi quantitative scale. The data from the macroscopical evaluation are shown in table 1 below. The data clearly demonstrate that skin healing was  
35    superior in the pigs treated with infliximab. This was also the case both for the superficial and deep muscle layers. Also bone healing was found to be more

pronounced in the infliximab treated pigs. The intraspinal scar adjacent to the nerves was also less hard and less attached to the underlying nerves in the infliximab treated pigs.

5

Table 1

Wound healing and scar formation in pigs subjected to laminectomy, with or without treatment of infliximab

	Skin	M sup.	M deep	M adh.	Bone h.	Scar con.	Scar adh.
Control	++	+	+	0	++	+	+
	++	++	++	(+)	++	++	++
	(+)	++	(+)	+	+	(+)	0
Infliximab	(+)	(+)	0	0	+	(+)	0
	(+)	0	0	0	+	(+)	0
	(+)	(+)	0	0	(+)	(+)	0

Skin (healing of the skin incision):

10 0 = perfectly healed, (+) = slight opening, + = pronounced opening,  
++ = infection

M sup. (healing of superficial muscle layer):

15 0 = perfectly healed, (+) = slight diastasis, + = clear diastasis,  
++ = hematoma or infection with loss of contact

M deep (healing of deep muscle layer):

20 0 = perfectly healed, (+) = slight diastasis, + = clear diastasis,  
++ = hematoma or infection with loss of contact

M adh. (adhesion of the muscle incision at weakest point):

0 = firm adhesion, (+) = weak adhesion, + = no adhesion

Bone h. (bone healing):

25 0 = laminectomy healed, (+) = more than 50% of the laminectomy  
healed, + = 25-50% of the laminectomy healed, ++ = less than 25% heal-  
ing

Scar con. (consistency of the scar adjacent to the intra spinal nerves):

0 = like water, (+) = soft gel, + = hard gel, ++ hard tissue

5 Scar adh. (adhesion of the scar to the nerves):

0 = no adhesion, (+) = gel-like adhesion, + = adhesion that is easily breakable, ++ = firm adhesion

Comparative example (not according to the invention)

10 Following a laminectomy of the lamina of the 4<sup>th</sup> lumbar vertebra either 0.15 ml of 20 ng/ml of recombinant rat TNF in distilled water or just 0.15 ml of distilled water was instilled in the laminectomy space. The wound was sutured and assessed after 1 week, 2 weeks regarding wound healing and scar tissue formation. There were 20 rats in total. Five rats were treated with TNF  
15 and five rats with only distilled water for each duration. Contrary to what could be expected the wound healing was significantly impaired in the rats exposed to TNF. The scar formation in the laminectomy space was significantly more pronounced in the TNF exposed rats, also contrary to what could be expected from the literature. The scar in the TNF exposed rats was also attached to the  
20 dura mater covering the spinal cord by adhesions. All observations were performed in a blinded fashion.

CLAIMS

1. Use of a substance that inhibits a pro-inflammatory cytokine for the production of a pharmaceutical composition for treatment of a wound by improving wound healing.  
5
2. Use according to claim 1, wherein said pro-inflammatory cytokine is selected from the group consisting of TNF, IL-1, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, GM-CSF, M-CSF, MCP-1, MIP-1, RANTES, ENA-78, OSM, FGF, PDGF, and VEGF.  
10
3. Use according to claim 1 or 2, wherein said pro-inflammatory cytokine is selected from the group consisting of TNF and IL-1.
4. Use according to any one of the claims 1 – 3, wherein said pharmaceutical composition is for treatment of posttraumatic tissue injury.  
15
5. Use according to claim 4, wherein said posttraumatic tissue injury is caused by surgery.  
20
6. Use according to any one of the claims 1 – 3, wherein said pharmaceutical composition is for treatment of thermic injury.
7. Use according to any one of the claims 1 – 3, wherein said pharmaceutical composition is for treatment of a wound resulting from a metabolic process due to reduced nutritional supply.  
25
8. Use according to claim 7, wherein said wound is a diabetic ulcer, a leg ulcer, a decubitus ulcer or a gastric ulcer.  
30
9. Use according to any one of the claims 1 - 3, wherein said pharmaceutical composition is for treatment of a wound resulting from exposure to a toxic compound.
10. Use according to any one of the claims 1 – 9, wherein said substance is a monoclonal antibody.  
35

11. Use according to claim 10, wherein said substance is selected from the group consisting of infliximab, CDP-571, D2E7 and CDP-870.

5           12. Use according to any one of the claims 1 – 9, wherein said substance is a soluble cytokine receptor.

13. Use according to claim 12, wherein said substance is etanercept.

10           14. Use according to any one of the claims 1 – 9, wherein said substance is a receptor antagonist.

15           15. Use according to any one of the claims 1 – 9, wherein said substance is an antisense oligonucleotide.

16. Use according to any one of the claims 1 – 9, wherein said substance is an MMP inhibitor selected from the group consisting of tetracyclines, chemically modified tetracyclines, Prinomastat, Batimastat, Marimastat, KB-R7785, TIMP-1, TIMP-2, adTIMP-1, and adTIMP-2.

20           17. Use according to any one of the claims 1 – 9, wherein said substance is an quinolones selected from the group consisting of Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin, and Temafloxacin.

25           18. Use according to any one of the claims 1 – 9, wherein said substance is a thalidomide derivate selected from the group consisting of CC-1088, CDC-501, CDC-801 and Linomide.

30           19. Use according to any one of the claims 1 – 9, wherein said substance is selected from the group consisting of prostaglandins, phosphodiesterase I, II, III, IV, and V-inhibitors, cyclosporin, pentoxifyllin derivatives, hydroxamic acid derivatives, melanin and melancortin agonists, and lazaroids.

35

20. Use according to any one of the claims 1 – 9, wherein said substance is a specific IL-1 $\alpha$  and/or IL-1 $\beta$  blocking substance.

21. Use according to any one of the claims 1 – 9, wherein said substance is a non-specific IL-1 $\alpha$  and/or IL-1 $\beta$  blocking substance.

22. Use according to any one of the claims 1 – 9, wherein said substance is lactoferrin or a peptide derived or derivable from lactoferrin.

23. Use according to any one of the claims 1 – 22, wherein said pharmaceutical composition is formulated for localized administration.

24. Use according to any one of the claims 1 – 22, wherein said pharmaceutical composition is formulated for systemical administration.

25. A method for improving wound healing wherein a therapeutically effective amount of a substance that inhibits a pro-inflammatory cytokine is administered to a patient in need of said treatment.

26. A method according to claim 25, wherein said pro-inflammatory cytokine is selected from the group consisting of TNF, IL-1, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, GM-CSF, M-CSF, MCP-1, MIP-1, RANTES, ENA-78, OSM, FGF, PDGF, and VEGF.

27. A method according to 25 or 26, wherein said pro-inflammatory cytokine is selected from the group consisting of TNF and IL-1.

28. A method according to any one of the claims 25 – 27, for treatment of posttraumatic tissue injury.

29. A method according to claim 28, wherein said posttraumatic tissue injury is caused by surgery.

30. A method according to any one of the claims 25 – 27, for treatment of thermic injury.

31. A method according to any one of the claims 25 – 27, for treatment of a wound resulting from a metabolic process due to reduced nutritional supply.

5           32. A method according to claim 31, for treatment of a diabetic ulcer, a leg ulcer, a decubitus ulcer or a gastric ulcer.

33. A method according to any one of the claims 25 – 27, for treatment of a wound resulting from exposure to a toxic compound.  
10

34. A method according to any one of the claims 25 – 33, wherein said substance is a monoclonal antibody.

35. A method according to claim 34, wherein said substance is selected from the group consisting of infliximab, CDP-571, D2E7 and CDP-870.  
15

36. A method according to any one of the claims 25 – 33, wherein said substance is a soluble cytokine receptor.

20           37. A method according to claim 36, wherein said substance is etanercept.

38. A method according to any one of the claims 25 – 33, wherein said substance is a receptor antagonist.  
25

39. A method according to any one of the claims 25 – 33, wherein said substance is an antisense oligonucleotide.

40. A method according to any one of the claims 25 – 33, wherein said substance is an MMP inhibitor selected from the group consisting of tetracyclines, chemically modified tetracyclines, Prinomastat, Batimastat, Marimastat, KB-R7785, TIMP-1, TIMP-2, adTIMP-1, and adTIMP-2.  
30

41. A method according to any one of the claims 25 – 33, wherein said substance is an quinolones selected from the group consisting of Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatiflox-  
35

acin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin, and Temafloxacin.

42. A method according to any one of the claims 25 – 33, wherein said  
5 substance is a thalidomide derivate selected from the group consisting of CC-1088, CDC-501, CDC-801 and Linomide.

43. A method according to any one of the claims 25 – 33, wherein said  
substance is selected from the group consisting of prostaglandins, phosphodi-  
10 esterase I, II, III, IV, and V-inhibitors, cyclosporin, pentoxifyllin derivatives, hydroxamic acid derivatives, melanin and melancortin agonists, and lazaroids.

44. A method according to any one of the claims 25 – 33, wherein said  
substance is a specific IL-1 $\alpha$  and/or IL-1 $\beta$  blocking substance.  
15

45. A method according to any one of the claims 25 – 33, wherein said  
substance is a non-specific IL-1 $\alpha$  and/or IL-1 $\beta$  blocking substance.

46. A method according to any one of the claims 25 – 33, wherein said  
20 substance is lactoferrin or a peptide derived or derivable from lactoferrin.

47. A method according to any one of the claims 25 – 46, wherein said  
substance is locally administered.

48. A method according to any one of the claims 25 – 46, wherein said  
25 substance is systemically administered.